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Mitochondrial Mechanisms of Redox Cycling Agents Implicated in Parkinson's Disease

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Abstract

Environmental agents have been implicated in Parkinson's disease (PD) based on epidemiological studies and the ability of toxicants to replicate features of PD. However, the precise mechanisms by which toxicants induce dopaminergic toxicity observed in the idiopathic form of PD remain to be fully understood. The roles of ROS and mitochondria are strongly suggested in the mechanisms by which these toxicants exert dopaminergic toxicity. There are marked differences and similarities shared by the toxicants in increasing steady-state levels of mitochondrial ROS. Furthermore, toxicants increase steady-state mitochondrial ROS levels by stimulating the production, inhibiting the antioxidant pathways of both. This review will focus on the role of mitochondria and ROS in PD associated with environmental exposures to redox-based toxicants.

Keywords

Parkinson's disease; Mitochondria; Reactive oxygen species; Redox-cycling; Pesticides; Antioxidants

Parkinson's disease

Parkinson's disease (PD) was first described in 1817 by James Parkinson in his paper titled “*An Essay on the Shaking Palsy*” (Parkinson 2002). In this essay, he describes the symptoms of the disease as “involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured.” (Parkinson 2002). In the clinic today PD is characterized by four major hallmarks: (1) rigidity, (2) resting tremor, (3) bradykinesia or a slowness of movements and (4) postural instability (Lang and Lozano 1998). Although the majority of symptoms are motor in nature, patients who are diagnosed from PD often also suffer from non-motor systems such as cognitive impairments, depression, sleep disturbances and gastrointestinal troubles (Ziemssen and Reichmann 2007).

PD currently affects over 1 million people in North America, is the second most common neurodegenerative disorder and the most common movement disorder (Lang and Lozano

1998; Shastri 2003). Older age is the primary risk factor for PD indicated by a 40-fold increase in prevalence in people between the ages of 55 and 85 (Fahn and Sulzer 2004). The etiology of PD involves a 60–70 % loss of dopaminergic (DAergic) neurons within an area of the midbrain termed the substantia nigra pars compacta (SNpc). The loss of DAergic neurons in the SNpc results in a decrease of DA release into the striatum resulting in changes in the nigrostriatal system cumulating in motor deficits (Lang and Lozano 1998). Specifically, the loss of DAergic neurons causes a decreased release of DA into the putamen which affects both the direct and indirect pathways of movement. DA generates an excitatory signal in the direct pathway via activation of DA1 receptors and an inhibitory signal in the indirect pathway via DA2 receptors. Thus, when released DA levels are decreased (due to loss of DA producing cells in the SNpc), there is hyperactivity in the inhibitory DA2/indirect pathway and decreased activity in the excitatory DA1/direct pathway resulting in the clinical manifestation seen in PD of an inability to initiate or control movement once initiated (Smith et al. 2011).

Another hallmark of PD is the formation of Lewy bodies which are fibrous formations found in the cytoplasm of DAergic neurons. Lewy bodies are thought to be formed from improper degradation of numerous proteins including α -synuclein and ubiquitin (Lang and Lozano 1998; Shastri 2003; Forno et al. 1996). Interestingly, Lewy bodies have been associated with other diseases in addition to PD and Lewy bodies have been found in regions of the brain outside of the SNpc (dorsal motor nucleus of the vagus, the nucleus basalis of Meynert, and the locus coeruleus) in PD patients (Spillantini et al. 1998). Therefore, the exact role of Lewy body formation in the pathogenesis of PD is still unknown, but much research is looking at their role in PD and other neurodegenerative diseases.

There are two main types of PD, a genetically linked and an idiopathic/sporadic form. The majority of patients with PD suffer from the idiopathic form of the disease (~85–90 %) while the remainder have the earlier onset genetically linked form (Fahn 2003). Genetic forms of PD have been linked to mutations in numerous genes including; α -synuclein, Parkin, Pink1, DJ-1 and LRRK2 (Dauer and Przedborski 2003; Lin and Beal 2006; Sato and Hattori 2011). Mutations in many of these genes have been associated with increased oxidative stress, decreased complex I activity, increased mitochondrial DNA damage and increased reactive oxygen species (ROS) production culminating in increased mitochondrial dysfunction and DAergic cell death (Sato and Hattori 2011; Abou-Sleiman et al. 2006; Henchcliffe and Beal 2008; Dawson and Dawson 2003). There is a large body of literature discussing genetics in PD and is discussed in-depth in other review articles (Abou-Sleiman et al. 2006; Henchcliffe and Beal 2008; Subramaniam and Chesselet 2013).

ROS production and PD, role of the mitochondria

For the idiopathic PD patients, the exact cause of DAergic cell death is still unresolved. Recent literature has suggested a role of ROS production in PD etiology. The purpose of this review is to provide a background on the role of mitochondria in ROS production and provide recent evidence indicating the role of ROS in PD etiology either through an increase in production (due to environmental or endogenous toxicants) or a decrease in detoxification and not to detail the first historical descriptions of specific discoveries.

ROS are defined as any oxygen species that are capable of independent existence and contain one or more unpaired electrons (Halliwell and Gutteridge 2007). ROS include oxygen (O_2) (though not typically thought as one), superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), hydroxyl radical ($HO^{\cdot}$) and peroxynitrite ($ONOO^-$) (Halliwell and Gutteridge 2007). ROS are formed from the gain or loss of an electron by multiple mechanisms throughout the cell. These include, but are not limited to, nitric oxide synthases (NOS), xanthine oxidase, NAD(P)H oxidase, heme containing proteins such as aconitase and hemoglobin, autoxidation with molecules like adrenalin, DA and flavin adenine dinucleotide (FAD), and electron leak from the electron transport chain (ETC) within the mitochondria (Halliwell and Gutteridge 2007; Chandel and Budinger 2007). Once formed, ROS can react with metals, other ROS, proteins, lipids, DNA, or antioxidant enzymes. Depending on the reaction, these interactions can result in activation of cell signaling pathways, oxidative damage, or the conversion of ROS into a non-reactive species such as water (H_2O). ROS are important for many signaling pathways, immune responses and the regulation of transcription factors (Chandel and Budinger 2007; Veal et al. 2007).

Mitochondria have long been known to be a major source of ROS production (Halliwell and Gutteridge 2007; Murphy 2009). ROS production is a natural by-product of the mitochondria's primary role in cells to produce adenosine triphosphate (ATP) using substrates generated by the tricarboxylic acid (TCA) cycle to feed electrons into the ETC. Because mitochondria produce a large amount of ATP, they are often thought of as the 'powerhouse' of the cell, however, mitochondria have other important cellular functions including fatty acid synthesis, the biogenesis of heme and iron-sulfur proteins, O_2 sensing, calcium (Ca^{2+}) homeostasis and the regulation of apoptosis (Collins et al. 2012; Tait and Green 2012). The ETC is composed of five subunits which transfer electrons from one complex to another to generate a proton (H^+) gradient (Fig. 1). The H^+ gradient is then utilized by ATP synthase (complex V) to convert adenosine diphosphate (ADP) into ATP. The presence of O_2 is required as the terminal acceptor for electrons, which will produce the non-toxic by-product H_2O . Electrons can leak from NADH dehydrogenase (complex I) or cytochrome C (complex III) and react with O_2 , and through one electron reduction, produce $O_2^{\cdot-}$ (Murphy 2009). It is estimated that $\sim 0.1\text{--}2\%$ of total mitochondrial O_2 consumption results in $O_2^{\cdot-}$ formation, however, the actual rates in vivo may be less (Murphy 2009; Boveris and Chance 1973). $O_2^{\cdot-}$ can spontaneously dismutate non-enzymatically or be actively converted into H_2O_2 through the enzymatic action of manganese superoxide dismutase (MnSOD) (Murphy 2009). Additional sources of H_2O_2 in mitochondria include enzymes such as monoamine oxidase (MAO), α -ketoglutarate dehydrogenases and aconitase (Murphy 2009; Turrens 2003).

To ensure ROS levels are maintained at physiological levels, endogenous antioxidants are present to delay or prevent oxidative damage to proteins, lipids and DNA (Gutteridge and Halliwell 2010). Antioxidants can be complex oxidizable molecules or simple thiols, they reside in multiple structures and organelles within a cell, and are common control elements for multiple biological processes (Gutteridge and Halliwell 2010; Jones and Go 2010). Within the cell there are the endogenous antioxidant systems of glutathione (GSH), thioredoxin/thioredoxin reductase/peroxiredoxin (Trx/TrxR/Prx) and catalase to maintain the redox status by converting H_2O_2 into H_2O (Fig. 1). When there is a disruption of the

antioxidant systems or redox circuits or there is an uncontrollable increase in ROS production, oxidative stress and/or damage may occur. Depending on the severity of this damage, it can result in impaired cellular functions potentially leading to cell death. Oxidative stress has been implicated in multiple diseases including PD, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), stroke, obesity, diabetes, aging and heart failure (Lin and Beal 2006; Baynes 1991; Cadenas and Davies 2000; Cherubini et al. 2005; Furukawa et al. 2004; Giordano 2005). Therefore, the intricate play between ROS production, detoxification, signaling and damage has been an area of great interest to researchers and the role of antioxidants in mitochondrial ROS detoxification in PD will be discussed in-depth later in the article.

With regard to ROS production and PD, postmortem brain tissue analysis has shown decreased complex I activity and an increase in 4-hydroxyl-2-nonenal (4-HNE) adducts, lipid peroxidation and DNA oxidation (all marker of increased oxidative stress) in patients with PD compared to age matched controls (Bove and Perier 2012; Floor and Wetzel 1998; Parker et al. 2008; Yoritaka et al. 1996). The cause of this increased oxidative stress and mitochondrial dysfunction in PD patients is still unknown. In a populationbased case–control study, Gorell et al. (1998) showed farming as an occupation was significantly associated with PD. Additionally, a metaanalysis conducted by Priyadarshi et al. (2001) found that living in a rural area and dinking well water may be a risk factor for developing PD. These epidemiological studies and others have linked pesticide exposure with development of sporadic PD, however, the link between rural living and drinking well water is still debated and more research is needed to define their exact roles in PD (Gorell et al. 1998; Priyadarshi et al. 2001; Dinis-Oliveira et al. 2006; Hertzman et al. 1990; Liou et al. 1997).

The exact cause of ROS-induced DA-specific cell death is also unknown but currently researchers hypothesize that the sporadic PD may be a result of a combination of genetic and environmental factors or a ‘multi-hit’ hypothesis (Cicchetti et al. 2009; Logroscino 2005). In support of this multi-hit hypothesis, it has been found that some pesticides and toxicants can act directly on DAergic neurons and generate mitochondrial or cellular ROS. Additionally, exposure to redox active metals like iron (Fe) and copper (Cu) or excessive accumulation of manganese (Mn) has been linked to the etiology of Parkinson's disease (Jomova and Valko 2011; Guilarte 2010; Graham 1984). Finally, it has been found that patients with PD have decreased endogenous antioxidant activities and levels (Bove and Perier 2012). This, combined with the increased basal ROS production in DAergic cells compared to other neuronal cells, would potentially increase mitochondrial or cellular ROS to pathological levels resulting in the pathogenesis of PD. All environmental links (increased metal, viral infection, increased Mn levels, etc.) to the generation of PD will not be discussed in this review. Instead this review will focus on the environmental ROS producing toxicants—methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat (PQ) and the endogenous ROS generator 6-hydroxydopamine (6OHDA).

Role of ROS producing toxicants and pesticides in PD

DA-specific toxicants and PD: MPTP

The idea of environmental compounds leading to DAergic cell death through mitochondrial ROS production was first demonstrated with a small group of young adult drug users who exposed themselves to the compound MPTP and developed the clinical symptoms of PD (Langston et al. 1983). It was later discovered that MPTP is highly lipophilic and thus can readily cross the blood brain barrier (BBB). Once in the brain, MPTP is metabolized by monoamine oxidase B (MAO-B) in the glial cells to produce the detrimental radical 1-methyl-4-phenylpyridinium (MPP⁺) (Heikkila et al. 1984; Nicklas et al. 1985). MPP⁺ is released by glial cells where it is readily taken up by the DA transporter into DAergic cells where it reacts with the NADH binding domain of complex I, increasing electron leak resulting in increased ROS production, specifically high levels of O₂⁻ (Heikkila et al. 1984; Nicklas et al. 1985; Cleeter et al. 1992; Hasegawa et al. 1990; Javitch et al. 1985). It has been noted that the reduction potential for MPP⁺ is approximately -1 V at physiological pH making it a poor redox cycling agent (Chacon et al. 1987). Despite the poor reduction potential, some literature has indicated that MPP⁺ is redox active in the presence of certain electron donors such as cytochrome 450 reductase, xanthine oxidase and aldehyde dehydrogenase to produce OH⁻ (Adams et al. 1992; Klaidman et al. 1993). It is hypothesized that MPP⁺ redox cycles by the reduction of MPP⁺ into the MPP radical which then cleaves H₂O₂ to produce OH⁻ and the parental MPP⁺ (Adams et al. 1992). However, the role of MPP⁺ in ROS production via redox cycling is still hotly debated and whether or not it can redox cycle within the mitochondria is still unknown. Despite the debate regarding the redox cycling mechanism, it is widely accepted that MPTP/MPP⁺ can generate high levels of DAergic cellular ROS through complex I inhibition in the mitochondria.

Since its discovery, MPTP has been widely used to produce DAergic-specific cell death resulting in PD symptoms in multiple animal models, including mice and non-human primates (Bove and Perier 2012). However, criticism has arisen regarding the relevance MPTP exposure to a general population to cause idiopathic PD. Nevertheless, the discovery of the toxic mechanisms of MPTP laid the groundwork for the environmental hypothesis of PD and to this day is used as a robust model to mimic PD in animals to better understand the mechanism of DAergic cell death and for the purpose of developing new therapeutics.

Endogenous DA-specific toxicants: 6OHDA

Similar to MPTP, 6OHDA is a neurotoxicant which directly affects DAergic cells due to its specificity to be taken up by the DA transporter. Once inside the DAergic cell, 6OHDA can exert its toxic effects through a cytosolic and a mitochondrial mechanism. Within the cytosol, 6OHDA promotes ROS production through the generation of quinones by an interaction with DA or with-OH via Fenton chemistry with iron (Blum et al. 2001). 6OHDA can be transported into the mitochondria where it inhibits complex I of the ETC resulting in increased ROS production, decreased ATP production and increased mitochondrial dysfunction (Blum et al. 2001; Deumens et al. 2002). However, unlike MPTP, 6OHDA cannot cross the BBB and therefore must be directly administered intracerebrally to yield its toxic effects making it a poor environmental-exposure model of PD (Blum et al. 2001).

Nevertheless, studies in postmortem brain tissue from humans and rats have found detectable levels of endogenous 6OHDA, indicating that 6OHDA may be a physiological endogenous neurotoxin (Curtius et al. 1974). Because of its specificity to induce cell death in catecholamine producing neurons and its potential role as an endogenous toxicant, 6OHDA is used as a neurotoxin to generate animal models of PD.

Pesticides and PD: rotenone

Since the discovery of ROS-induced DAergic toxicity by the above-mentioned chemicals, researchers have begun asking if there are other environmental toxicants linked to PD development. Interestingly, a few pesticides have been identified to produce PD symptoms in animals and epidemiological studies have linked exposure to some of these pesticides or herbicides, alone or in combination to increase the risk for developing PD. One such pesticide which has been linked to PD in epidemiological studies is rotenone (Dhillon et al. 2008). Rotenone is a naturally occurring toxin found in the root of the *derris* and *lonchocarpus* plants (Haller et al. 1942). Rotenone is a highly lipophilic and has been used as a pesticide spray and has also been used to kill fish in lakes and reservoirs (Haller et al. 1942; Uversky 2004). Like MPTP and 6OHDA, rotenone's toxicity is a result of its ability to bind and inhibit complex I and thus increase $O_2^{\cdot-}$ levels and deplete ATP synthesis (Sheeran et al. 2010). Rats which have been chronically treated with rotenone develop a significant loss of nigrostriatal DAergic neurons and develop Lewy bodylike inclusions (Betarbet et al. 2000, 2002). Additionally, primary midbrain cells exposed to rotenone have increased oxidative damage resulting in caspase-3-dependent cell death (Ahmadi et al. 2003). As a result of these in vivo and in vitro studies, rotenone is often used as a cellular or animal model in pesticide-based PD development.

Pesticides and PD: PQ

A herbicide which has gained attention for having an epidemiological link to an increased risk for PD development is PQ (Dinis-Oliveira et al. 2006; Hertzman et al. 1990; Liou et al. 1997; Tanner et al. 2011). PQ is one of the most widely used herbicides in the world and is primarily used in the USA as weed control in orchards and plantations (Drechsel and Patel 2008). PQ is a member of the bipyridyl herbicide family due to its two covalently linked pyridine rings (Drechsel and Patel 2008). Of interest, the structure of PQ is very similar to MPP⁺; however, despite the structural similarities, MPP⁺ and PQ have very different mechanisms of action. PQ's toxicity is a result of its ability to redox cycle; accepting an electron from a donor creating a PQ radical (PQ^{•+}) which in turn reduces O_2 to produce $O_2^{\cdot-}$ and the parental PQ compound (Figs. 1, 2). PQ has a reduction potential of -0.450 V which is biologically important due to NADP⁺/NADPH and NAD⁺/NADH having reduction potentials at -0.324 and -0.320 , respectively (Drechsel and Patel 2008). This is significantly higher than the redox potential of MPP⁺ indicating a higher availability to redox cycle (as discussed previously). Therefore, under normal physiological conditions PQ can accept electrons from redox couples including enzymes which transfer electrons from NAD(P)H. These include but are not limited to NADPH cytochrome c reductase, nitric oxide synthase, NADPH oxidase, and complex I and III of the ETC (Bonneh-Barkay et al. 2005; Castello et al. 2007; Clejan and Cederbaum 1989; Day et al. 1999; Drechsel and Patel 2009; Fukushima et al. 1993).

Of interest with regards to mitochondrial ROS production and PD, PQ can pass into the mitochondrial matrix where it redox cycles with complex I and III of the ETC. Fukushima et al. (1993) indicated that PQ can redox cycle with complex I when present in millimolar concentrations. Subsequently, Drechsel and Patel (2009) discovered that PQ accepts electrons from complex III of the ETC to form PQ^+ at micromolar concentrations. Due to the high utilization of O_2 within the mitochondria, PQ^+ rapidly produces $O_2^{\cdot-}$ and the parental PQ within isolated mitochondria and in primary rat midbrain cultures (Castello et al. 2007). Thus, as long as electrons and O_2 are present, PQ can continuously redox cycle within the mitochondria generating a high level of mitochondrial $O_2^{\cdot-}$ which is rapidly converted to H_2O_2 via MnSOD and diffuses into the cytosol increasing cellular oxidative stress (Drechsel and Patel 2008, 2009).

Interestingly, people exposed to lethal doses of PQ typically die from lung toxicity (due to the high O_2 levels present in the lung); however, significant damage to the brain has been observed (Grant et al. 1980; Hughes 1988; Smith 1982). Epidemiological studies have indicated an increased risk for developing PD after chronic exposure to PQ (Dinis-Oliveira et al. 2006; Hertzman et al. 1990; Liou et al. 1997; Tanner et al. 2011). In animal models, mice treated chronically with direct injection of PQ into the striatum had a loss of DAergic neurons within the substantia nigra (SN) and decreased striatal DA nerve fibers (Brooks et al. 1999; McCormack et al. 2002). Additionally, mice receiving multiple injections of PQ had a reduction in motor activity correlating with a dosedependent decrease in DAergic nerve fibers in the striatum (Brooks et al. 1999). The ability of PQ to cross the BBB is limited with systematic exposure, however, pretreatment with valine or phenylalanine (blockers of neutral amino acid transport into the brain) prevents PQ-induced neurodegeneration in mice, indicating PQ can cross the BBB in animal models, but at a much lower concentration than MPTP (McCormack et al. 2002; Shimizu et al. 2001).

As an herbicide, PQ is often mixed in preparation with another bipyridyl herbicide diquat (DQ) (Price et al. 1995). Similar to PQ, DQ's mechanism of action is the generation of high levels of $O_2^{\cdot-}$ through redox cycling. DQ will undergo a single electron addition to form the DQ radical which is then cycled back to the parental DQ compound through a reaction with O_2 producing $O_2^{\cdot-}$ (Jones and Vale 2000). It is important to note that PQ produces radical formation at a greater rate within the mitochondria and is more toxic in lung, liver and kidney tissue than DQ (Tomita 1991). It has been noted that exposure to DQ results in damage to the central nervous system and it was reported in a case study that a farmer exposed to high levels of DQ developed severe Parkinsonism syndrome (Sechi et al. 1992). Mice exposed to DQ alone do not have DA depletion in the striatum but do have some motor impairments suggesting that radical formation by DQ alone may decrease DA turnover resulting in mild motor impairments (Karuppagounder et al. 2012). Therefore, DQ alone has very limited DAergic toxicity, however, when combined with another redox cycling agent (such as PQ) the combination will generate a significant increase in ROS production than either agent alone cumulating in the increase DA depletion.

PQ is also often mixed with the manganese-containing ethylene-bis-dithiocarbamate fungicide maneb, to treat numerous plant diseases. The combination of PQ/maneb has been linked to the development of PD in epidemiological studies (Costello et al. 2009). It has

been shown that mice treated with both maneb and PQ have an increase in nigrostriatal cell death and deficits in motor behavior combined to either agent alone (Subramaniam and Chesselet 2013; Morato et al. 1989; Kachroo et al. 2010). Similar to DQ, maneb alone has very limited toxicity but when combined with PQ will increase the toxicity of PQ within the brain (Barlow et al. 2005; Roede et al. 2011). In isolated brain mitochondria and primary mesen-cephalic cultures, it has been demonstrated that maneb inhibits complex III of the ETC resulting in increased ROS production and impaired mitochondria function (Domico et al. 2006; Zhang et al. 2003). Both maneb and PQ react with complex III of the ETC and, therefore it is hypothesized that the combined treatment of both toxicants exacerbates the ROS production of PQ at complex III, causing increased mitochondrial ROS release, mitochondrial dysfunction and cell death. Additionally it has been demonstrated that maneb can deplete thiols of the antioxidant thioredoxin (Roede and Jones 2014). Depletion of either the cytosolic or mitochondrial thioredoxin system has been linked to an increase in mitochondrial dysfunction, ROS production and resulting cell death when DAergic cells are exposed to low levels of PQ (Lopert et al. 2012). Therefore, when cells or animals are exposed to PQ and maneb, the increased ROS production at complex III with the decreased Trx/TrxR activity leads to an increase in cell death (Roede and Jones 2014; Lopert et al. 2012). Other mechanisms of action have been hypothesized (alkylation, decreased glutathione levels, decreased antioxidant activity, neuronal excitotoxicity) and hence more research is needed to determine all the mechanisms of action that generate this synergistic and epidemiologically important effect (Roede et al. 2011).

Antioxidants role with PD-redox cycling agents

Glutathione (GSH)

As stated previously, endogenous antioxidants are present to maintain the delicate balance of ROS production for cellular processes and detoxification to prevent oxidative stress. Within brain mitochondria and DAergic cells, MnSOD will dismutate $O_2^{\cdot -}$ into H_2O_2 which can then be reduced to water through the action of the GSH and/or the Trx/TrxR/Prx system. Of these two antioxidant pathways, the GSH system has been studied the most due to it being the most abundant non-protein thiol in cells (Dickinson and Forman 2002; Zeevalk et al. 2008). When high levels of H_2O_2 are present, glutathione peroxidase (GPx) will detoxify H_2O_2 into H_2O . GPx is then kept in the reduced form by GSH, which becomes oxidized and forms glutathione disulfide (GSSG). GSSG is recycled back to GSH through the action of glutathione reductase (GR) by converting NADPH to $NADP^+$ (Fig. 1). GSH can also nonenzymatically scavenge ROS and modify protein sulfhydryl groups by either directly reducing the sulfenic acids or by forming a protein with a mixed disulfide and further reduction (Martin and Teismann 2009). GSH can also form conjugates with electrophilic compounds through the action of glutathione-s-transferase (GST) which can then be targeted for degradation (Martin and Teismann 2009). In addition to its antioxidant actions, GSH has also been linked to DNA synthesis and repair, immune function, protein synthesis, amino acid transport and may potentially have some neurotransmitter functions (Bains and Shaw 1997; Lomaestro and Malone 1995; Shaw et al. 1996).

GSH levels decrease as a person ages, suggesting that age-related GSH decreases may underlie a number of changes associated with normal aging and may be involved with the onset of various age-related diseases, including PD (Bains and Shaw 1997; Lang et al. 1989). Indeed, patients with PD have a 40 % decrease in GSH in the SN compared to controls (Perry et al. 1982; Sian et al. 1994; Sofic et al. 1992). The decrease in GSH levels may be due to a decrease in GSH synthesis, through decreased glutamate cysteine ligase (GCL) activity, or a decrease in the recycling of GSSG to GSH through decreased GR activity. Interestingly, mice with the modifier subunit of GCL (GCLM) knocked out, which results in ~60 % depletion of brain GSH, are healthy and fertile and show no change in striatal complex I activity, DA levels, 3-nitrotyrosine/tyrosine ratios, or aconitase activity (Liang et al. 2013; Yang et al. 2002). However, these mice are more susceptible to PQ toxicity (Liang et al. 2013). These data indicate that decreased GSH levels (which occur naturally as one ages) increase susceptibility to PQ-induced oxidative stress which has been linked in epidemiological studies to increase the risk of developing PD (Dinis-Oliveira et al. 2006; Liang et al. 2013).

Thioredoxin/peroxiredoxin system

Similar to the GSH system, the Prx/Trx/TrxR antioxidant system converts H_2O_2 into H_2O . As outlined in Fig. 1, Prx detoxifies H_2O_2 into water and is kept in a reduced state through the oxidation of Trx. Trx is then reduced through oxidation of TrxR. TrxR is kept in a reduced state by the conversion of NADPH into NADP^+ (Fig. 1). Unlike GPx, Prxs are thiol-dependent peroxidases which employ cysteine residues at the reaction site resulting in Prxs having a slower reaction rate for H_2O_2 than Gpx (Halliwell and Gutteridge 2007). However, Prxs are present in higher concentrations and have a higher affinity for H_2O_2 than Gpx indicating that they likely play a critical role in H_2O_2 detoxification (Halliwell and Gutteridge 2007).

Unlike the GSH system, knockout of the mitochondrial Trx or TrxR (Trx2/TrxR2) is embryonically lethal and death occurs around the time of mitochondrial maturation, between days 10 and 13 (Conrad et al. 2004; Hanschmann et al. 2013; Nonn et al. 2003). These knockout mice exhibit large amounts of oxidative stress in the heart, another highly metabolic and ROS producing organ, and Trx2 knockout embryos are not viable for cell culture (Conrad et al. 2004; Hanschmann et al. 2013; Nonn et al. 2003). Trx2/TrxR2 expression is higher in organs with a high mitochondrial content, such as the heart, brain, skeletal muscle, kidney and liver (Chen et al. 2002). Specifically in the brain, the strongest expression has been found in areas exhibiting the most severe oxidative stress with prominent expression within the SNpc (Rybnikova et al. 2000). Drechsel and Patel (2010) found that the Trx/TrxR system and not the GSH system is the main H_2O_2 detoxifying pathway in isolated brain mitochondria. Energized brain mitochondria consumed less H_2O_2 when the Trx/TrxR/Prx system was inhibited compared to inhibition of the GSH pathway (Drechsel and Patel 2010). Additionally, in a cellular model, it was shown that DAergic cells with mitochondrial TrxR knock down had synergistic increases in mitochondrial dysfunction, H_2O_2 production and cell death when exposed to PQ (Lopert et al. 2012). Interestingly TrxR inhibition plus PQ-induced cell death or loss of TH positive neurons in animals can be reversed when cells or animals are treated with a cell permeable catalytic antioxidant

indicating the role of ROS production and oxidative stress in PQ toxicity (Lopert et al. 2012; Callio et al. 2005; Day et al. 1995; Kulich et al. 2007; Patel and Day 1999). As mentioned previously, maneb can deplete thiols on Trx and combined with PQ treatment leads to potentiate the neurotoxicity of PQ (Roede and Jones 2014). Together this data indicates when mitochondrial antioxidants are depleted and DAergic cells have increased intracellular/intramitochondrial ROS production due to the redox cycling agents PQ or DQ, there is an increase in DAergic cell death as mimicking what is observed in PD.

Concluding remarks

To conclude, mitochondrial dysfunction and oxidative stress have been implicated in the pathogenesis of PD in both animal and human studies. It has been shown within the SNpc in postmortem tissue in PD patients that there is a (1) decrease in GSH levels and an increase in GSSG levels, (2) decrease in complex I activity, (3) increase in ROS production and (4) increase in mitochondrial dysfunction (Perry et al. 1982; Sian et al. 1994; Sofic et al. 1992). Decreased complex I activity has also been found in the plasma of patients with PD and, therefore complex I activity or GSH/GSSG levels may be a potential bio-marker for PD screening (Parker et al. 1989, 2008; Haas et al. 1995; Yoshino et al. 1992). Additionally, epidemiological studies have implied an increased risk of PD development in people exposed to pesticides or herbicides, specially the herbicide PQ whose mechanism of action is to produce high levels of ROS through mitochondrial redox cycling. Using pesticides and toxicants that generate high levels of mitochondrial ROS (such as PQ, 6OHDA or MPTP), researchers have been able to generate robust models of PD in multiple species which mimic some of the symptoms observed in PD patients (decreased GSH/increased GSSG levels, increased mitochondrial dysfunction and increased ROS production) (Betarbet et al. 2000, 2002). Antioxidant pathways utilized to detoxify ROS decrease as one ages, and thus it has been hypothesized (as outlined in Fig. 3) that the combination of age-related decreases in antioxidant capability with a lifetime of low-level exposure to redox cycling herbicides and increased susceptibility of DAergic neurons to oxidative stress due to DA breakdown into reactive quinones and semi-quinones interact resulting in increased DAergic cell death within the SNpc resulting in the pathogenesis of idiopathic PD.

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Fig. 1.

Schematic of mitochondrial production of ATP and ROS. The primary function of mitochondria is the production of ATP by the generation of the H^+ gradient through the passage of electrons (e^-) through the ETC (complex I–V). Electrons can leak from the ETC to produce $O_2^{\cdot -}$ which is dismutated to H_2O_2 by the action of MnSOD. H_2O_2 is then detoxified to H_2O by either the GSH or Prx/Trx antioxidant systems

Fig. 2.

Redox cycling mechanism of PQ. The parental PQ (PQ²⁺) compound will be converted to the PQ radical (PQ^{·+}) in the presence of an electron (e⁻). PQ^{·+} will react with oxygen (O₂) to generate superoxide (O₂^{·-}) and PQ²⁺ and thus in the presence of electrons and O₂, PQ can continually redox cycle

Fig 3.

Simplified summary environmental causes of PD. It is hypothesized that environmental factors such as MPTP and rotenone and redox cycling pesticides such as PQ and DQ increase ROS production within the mitochondria of DAergic cells. Increased levels of mitochondrial ROS, paired with age-related decreases in antioxidant function further potentiate mitochondrial dysfunction culminating in increased mitochondrial and cellular oxidative/nitrosative stress. Sustained elevated levels of ROS lead to oxidative damage to proteins, lipids and DNA resulting in increased DAergic cell death within the SNpc